MISSOURI DEPARTMENT OF NATURAL RESOURCES
AIR AND LAND PROTECTION DIVISION
ENVIRONMENTAL SERVICES PROGRAM
Standard Operating Procedures

SOP #: MDNR-WQMS-110 EFFECTIVE DATE: July 28, 2003

SOP TITLE: Instrument Calibration and Fluorometric Determination of Chlorophyll a Using the Turner Designs Fluorometer (Model TD-700).

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SUMMARY OF REVISIONS: Revisions were made to apply to the new Turner Designs TD-700 model fluorometer. Corrections were made to Section 6.2.3 and the formulas in Section 6.5.

APPLICABILITY: The procedures outlined in this SOP apply to all ESP personnel who perform chlorophyll a analysis using the Turner Designs TD-700 fluorometer.

DISTRIBUTION: MDNR Intranet
ESP SOP Coordinator

RECERTIFICATION RECORD:

Date Reviewed				
Initials				

1.0 SCOPE AND APPLICABILITY

1.1 A major water quality effect of nutrient pollution into waterways is nuisance levels of algae growth. Chlorophylls a, b, and c are pigments commonly found in phytoplankton and periphyton. Chlorophyll a forms about 1 to 2% of the organic material present in algae and is widely used for making estimates of algal biomass. Because of the rapidity and relative ease of measuring chlorophyll a concentration, this is an especially useful tool in water quality assessment.

1.2 Methods for determining chlorophyll a include spectrophotometric and fluorometric techniques. The fluorometric method for chlorophyll a analysis has several advantages over spectrophotometric methods. It is more sensitive to low chlorophyll concentrations allowing smaller samples to be analyzed; it is faster and is not dependent on critical wavelength settings; it is not as sensitive to turbidity and is not critically dependent on cuvette handling and matching. The main disadvantage is that only chlorophyll a, or a total of all chlorophylls (not chlorophyll b or chlorophyll c individually), can be determined fluorometrically. In addition, fluorometer calibration depends on the use of pure chlorophyll a standards with a properly calibrated spectrophotometer. For chlorophyll a analysis in freshwater algae, it is necessary to correct for chlorophyll b and pheophytin a (a breakdown product of chlorophyll a) values. Optical filters in the TD-700 are designed to correct for chlorophyll b. Pheophytin a correction is covered in section 6.4.

1.3 The procedures in this SOP cover methods for chlorophyll a analysis using the Turner Designs TD-700 fluorometer and are applicable to staff who operate the unit for chlorophyll a analysis and handle chlorophyll a samples and supplies.

2.0 SUMMARY OF PROCEDURES

These procedures generally follow Standard Methods for the Examination of Water and Wastewater 20th Edition, Section 10200 H. Known amounts of surface water volume for phytoplankton or surface area for periphyton are filtered and extracted for chlorophyll a analysis. This analysis with the Turner Designs TD-700 fluorometer (Appendix A) requires preparation of standards, instrument calibration, fluorometer readings, correction for pheophytin a, and final calculations.

3.0 SPECIAL CONSIDERATIONS

3.1 Refer to MDNR-WQMS-015 for sample collection and field handling of chlorophyll a samples. Refer to MDNR-WQMS-207 for laboratory extraction procedures for chlorophyll a analysis.

3.2 Because chlorophyll is easily broken down in the presence of light, conduct all handling and analysis of samples in subdued light and completely cover all glassware used for storing chlorophyll standards with foil.

3.3 Keep all primary chlorophyll standards refrigerated during storage.

3.4 Because skin secretions break down chlorophyll, wear nitrile or rubber gloves while handling and analyzing samples. Also wear protective gloves when handling fluorometer optical filters and lamp during installation.

3.5 Thoroughly triple rinse all reusable materials coming into contact with chlorophyll a samples and standards with buffered deionized water followed by a triple rinse with 95% ethanol.

3.6 To prevent scratching cuvettes when wiping dry, use laboratory tissues such as Kimwipes.

3.7 Because settings on the TD-700 tend to drift over time, frequently check calibration using the solid secondary standard (Appendix C). Check calibration with primary standards if necessary. ESP uses the Multi-Optional Raw Fluorescence method of calibration and analysis because this method provides more stable readings.

4.0 HEALTH AND SAFETY REQUIREMENTS

Wear personal protective equipment (PPE) including protective gloves and safety glasses. Because the ethanol contains toxic denaturing agents and is flammable, refer to the Material Safety Data Sheet for health and safety requirements when handling denatured ethanol.

5.0 SUPPLIES AND EQUIPMENT

- Turner Designs TD-700 fluorometer
- spectrophotometer
- glass cuvettes with adapter
- pure chlorophyll, approximately 1 mg
- volumetric flask, 1 L
- volumetric flasks (3-5), 100 ml
- aluminum foil
- solid secondary standard
- pipettes
- buffered water (saturated $MgCO_3$ solution in deionized water)
- 95% buffered ethanol (95% research grade ethanol, 5% buffered water)
- 5 N HCL

- dropper
- rubber or nitrile gloves
- logbook

6.0 PROCEDURES

6.1 Primary standard preparation

6.1.1 Prepare chlorophyll a super stock solution of approximately 10 mg/L by pouring the 1 mg of pure chlorophyll a into a 100 ml volumetric flask. Use 95% buffered ethanol solution to rinse the chlorophyll a ampule of all its contents into the flask. Using 95% buffered ethanol, fill the flask to the 100 ml level, cap, and mix. Cover the super stock flask completely with foil to prevent light from reaching the solution, place in refrigerator and allow the raw chlorophyll a to dissolve at a minimum overnight.

6.1.2 Using the spectrophotometer, measure the absorbance (ABS) of the super stock through a 1 cm^2 cell at 664 nm and 750 nm. Multiply the difference of the two readings by 11 for the actual concentration of chlorophyll a (Ca) in mg/L, which should typically range between 11 and 14 mg/L.

Ca (mg/L) = 11.0 X [ABS (664nm) - ABS (750nm)]

6.1.3 Prepare at least three chlorophyll a primary standards that should bracket actual concentrations anticipated for analysis by diluting superstock with 95% ethanol in 100 ml volumetric flasks. Suggested sets of dilutions are as follows assuming the super stock is 10 mg/L:

approximate Ca (µg/L)	volume of super stock
2.5	25 µl
10	100 µl
25	250 µl
50	500 µl
100	1 ml
500	5 ml

6.1.4 Determine the actual concentration of the primary standards in µg/L by multiplying the targeted concentration by:

$$\frac{\text{Actual Ca of super stock (mg/L)}}{\text{10 mg/L}}$$

For example, if the super stock solution is actually 12.5 mg/L, the actual concentration of the dilution targeted at 25 µg/L will be 31.25 µg/L.

6.1.5 Record superstock and primary standard concentrations in logbook.

6.1.6 Because primary standards are good only for a few months, they should be replaced as necessary and labeled with the preparation date.

6.2 TD-700 setup and calibration (6.2.2 to 6.2.8 from TD-700 Laboratory Fluorometer Operating Manual)

6.2.1 By following the TD-700 Laboratory Fluorometer Operating Manual, correctly install filters, filter cylinder, (Appendices B and D) and lamp.

6.2.2 Connect the power supply and turn on the TD-700 and allow adequate time for the unit to warm up. The Operating Manual suggests a ten-minute warm up period that is automatically timed by the instrument, however more warm up time seems to make the readings more stable.

6.2.3 On the TD-700 display, the HOME screen should be displayed after the warm up period. From the HOME screen, press <ENT>, press <1> for Setup, and press <1> again for Mode. Use the scroll key <↔> to choose "Multi-Optional Mode". Press <ESC> to return to the previous screen, then press <2> to choose the calibration procedure. Use the <↔> to choose "Raw Fluor" for the Raw Fluorescence calibration procedure. Press the <ESC> twice to return to the Setup/Cal screen.

6.2.4 To access the calibration sequence, press <2> from the Setup/Cal screen. The Multi-Optional-Raw Fluorescence calibration sequence will appear.

6.2.5 By following the Operating Manual, make sure the cuvette adapter is in place in the sample chamber (Appendices C and D) and fill a clean cuvette with a sample that is about 80% of the maximum concentration to be read. It is not necessary to know the exact concentration since it is used to set the optimal instrument sensitivity and range. Wipe the outside of the cuvette dry and insert it into the adapter in the sample chamber. Press <ENT> to proceed.

6.2.6 If the sample is 80% of the maximum concentration you wish to read, accept the default value of 800 by pressing <1>. The instrument will set the sensitivity so that the standard reads approximately 80% of the maximum range. If a reading of 800 is not acceptable, press<9> to change the value. If you assign a higher value to your sample, you will decrease the maximum sample concentration that can be read and increase the instrument's sensitivity and resolution. If you assign a lower value to your sample, you will increase the maximum sample concentration that can be read and decrease the instrument's sensitivity and resolution. Key in the desired number and press <ENT>, then press <1>.

6.2.7 The TD-700 will now set the sensitivity as indicated by SENS FACTOR, based on the final sample value accepted. Once the sample is set, the unit asks whether you want to run a blank. Press <1> to have the blank subtracted.

6.2.8 Fill a clean cuvette with the blank (95% buffered ethanol), wipe the outside of the cuvette dry, insert it into the cuvette adapter in the sample chamber, and close the chamber. Press <ENT>. Allow the reading to stabilize and press <0>. The instrument will read the blank and automatically return to the HOME screen.

6.2.9 Fill a clean cuvette with a sample of the primary standard of the lowest concentration. Wipe the outside of the cuvette dry, insert it into the cuvette adapter, and close the sample chamber. Once the reading has stabilized (no longer than 90 seconds), record it in the logbook as R_b (before acidification). Add two drops of 5.0 N HCl and record the reading once it stabilizes (no longer than 90 seconds) as R_a (after acidification).

6.2.10 Repeat Step 6.2.9 with the remaining primary standards.

6.2.11 Determine the r value which is the ratio of R_b:R_a and record in the logbook. Ideally this value should be 1.6 to 1.8. If the r value falls outside this range, contact the WQMS supervisor for further instructions.

6.2.12 Determine the Fs factor, which is the ratio of the actual concentration of the primary standards to the raw fluorescence values before acidification (R_b).

6.3 Sample analysis

6.3.1 Turn on the TD-700 and allow adequate time for the instrument to warm up.

6.3.2 Set the blank by inserting a cuvette filled with 95% buffered ethanol into the cuvette adapter in the sample chamber. From the HOME screen, press <0> and then press <1> as prompted. During multiple readings, periodically check the blank cuvette and reset if necessary.

6.3.3 Prior to analysis, remove the cuvette adapter, insert the solid secondary standard (Appendix C), and record low and high readings of the standard as instructed in the operating manual. Readings should be consistent from one analysis day to the next. If not, check readings of primary standards and consider recalibration. After secondary standard readings, replace cuvette adapter.

6.3.4 Set up the logbook to record sample numbers, volume (ml for phytoplankton) or area (cm^2 for periphyton) filtered, volume of extract (95% ethanol solution) added, dilution factor (see Step 7.3.6), and R_b and R_a values. Also record date of analysis, r-value, Fs value, and any other important information. The TD-700 may also be connected to a computer for receiving and recording readings and making calculations.

6.3.5 Make sure the extract is thoroughly mixed in its container by gently swirling. Transfer enough of the extract to a clean cuvette to almost fill it (approximately 8 ml). Wipe the outside of the cuvette dry and insert it into the adapter in the sample chamber. Close the chamber lid. Once the fluorescence reading has been established, record it in the logbook and/or send it to the computer by pressing<*>. This reading is the R_b value.

6.3.6 If the display indicates "OVER", the concentration in the cuvette is too high for the instrument to read. If this happens, dilute the sample with 95% ethanol. Measure a volume of extract from the original cuvette and a volume of 95% ethanol to dispense into another clean cuvette. Mix the contents by holding a clean piece of Parafilm over the top and inverting three or four times. Record the dilution factor (Df) as the percent represented as decimal value. For example, the Df of a sample that was diluted to one forth (25%) of its original strength will be recorded as 0.25. If no dilution is required, record 1.0 as the dilution factor.

6.4 Correction for pheophytin a

To correct for pheophytin a, add two drops of 5.0 N HCl to the cuvette. After the reading has stabilized for no longer than 90 seconds, record the value in the logbook and/or send it to the computer by pressing <*>. This is the R_a value.

6.5 Final calculations (may be done automatically by computer)

6.5.1 Calculate the concentrations of chlorophyll a (Ca) and pheophytin a (Pa) in the extraction cuvette with the following equations:

$$Ca\ (vial) = Fs[r/(r-1)](R_b-R_a)/Df \text{ in } \mu g/L$$

$$Pa\ (vial) = Fs[r/r-1)]r(R_a-R_b)/Df \text{ in } \mu g/L$$

6.5.2 In phytoplankton samples, to correct for volume filtered and volume of 95% ethanol used for extraction, multiply the concentration in the vial by the volume of the 95% ethanol (ml) divided by the volume of sample filtered (ml).

The final equations to determine chlorophyll a and pheophytin a in phytoplankton are as follows:

$$\text{Ca } (\mu g/L) = \frac{Fs[r/r-1)](R_b-R_a) \times \text{vol. extract (ml)}}{\text{vol. filtered (ml)} \times Df}$$

$$\text{Pa } (\mu g/L) = \frac{Fs[r/r-1)]r(R_a-R_b) \times \text{vol. extract (ml)}}{\text{vol. filtered (ml)} \times Df}$$

6.5.3 In periphyton samples, to correct for area sampled and volume of 95% ethanol used for extraction, multiply the concentration in the vial by the volume of 95% ethanol used and divide by the number of cm^2 filtered and divide by 100. (The '100' in the denominator provides a conversion factor for mass, volume, and area values)

$$\text{Ca } (mg/m^2) = \frac{Fs[r/r-1)](R_b-R_a) \times \text{vol. extract (ml)}}{\text{area filtered } (cm^2) \times Df \times 100}$$

$$\text{Pa } (mg/m^2) = \frac{Fs[r/r-1)]r(R_a-R_b) \times \text{vol. extract (ml)}}{\text{area filtered } (cm^2) \times Df \times 100}$$

7.0 REFERENCES

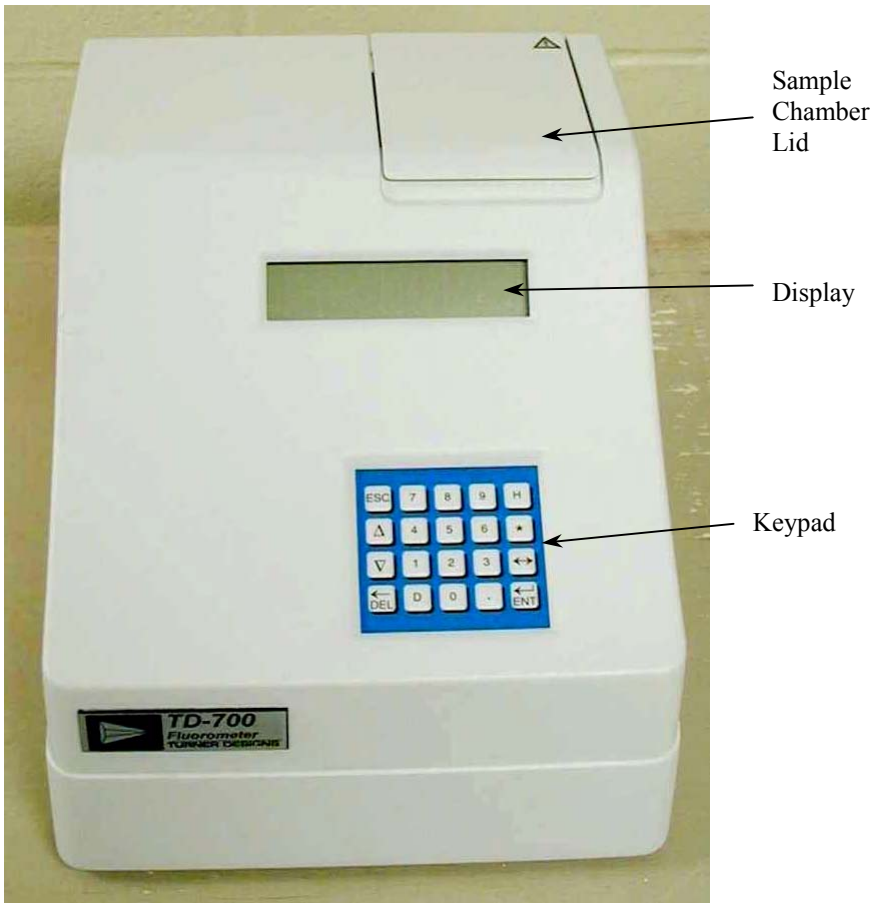
Standard Methods for the Examination of Water and Wastewater 20th Edition, Section 10200 H.

MDNR Environmental Services Program, MDNR-WQMS-015, *Sample Collection and Field Handling Procedures for Chlorophyll Analysis of Surface Water Samples*

MDNR Environmental Services Program, MDNR-WQMS-207, *Laboratory Extraction Procedure for Chlorophyll Analysis of Surface Water Samples to be Analyzed by Fluorometric or Spectrophotometric Techniques*

1999. TD-700 Laboratory Fluorometer Operating Manual. Turner Designs, Sunnyvale, CA. 54 pp.

Appendix A



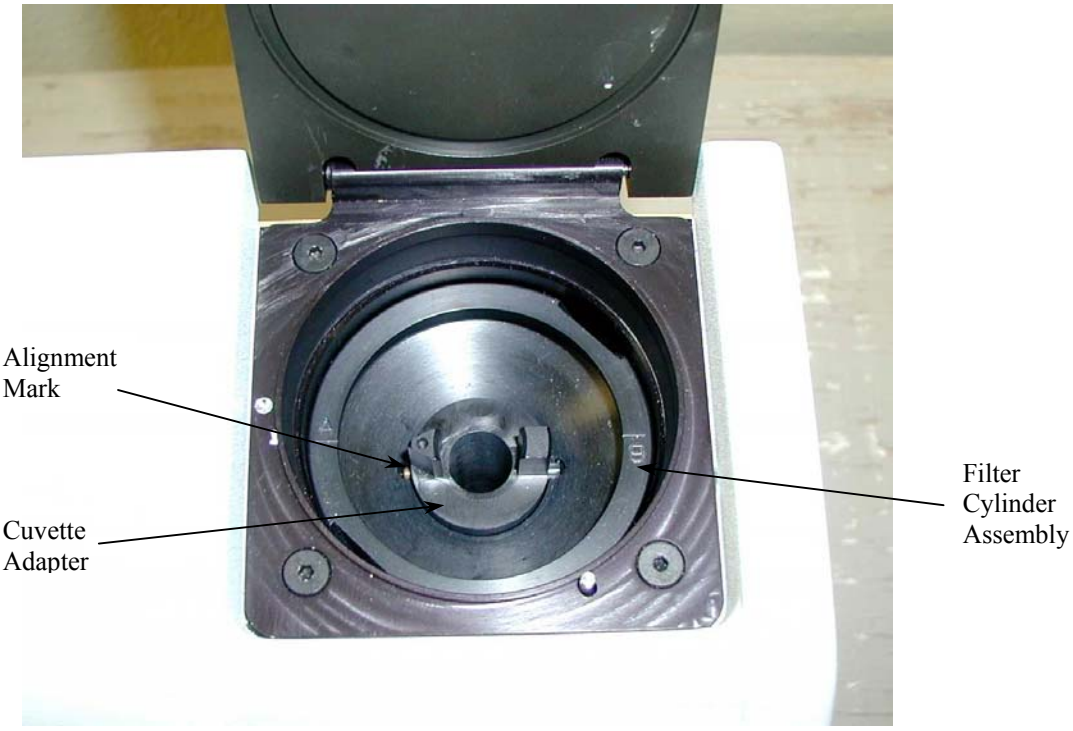
Turner Design TD-700 Laboratory Fluorometer

Appendix B

Filter Cylinder Assembly

Appendix C

Appendix D



Sample Chamber